Of systems and cancer

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2014

English summary

It has long been known that cancer has aberrant metabolic properties compared to healthy cells, including, famously, the tendency to forgo oxidative phosphorylation and produce lactate instead, even when oxygen is present. In the study of these properties, traditional metabolism-only and regulation-only approaches have shed some light on the matter, but no single explanation has covered all aspects of the Warburg effect — let alone all hallmarks of cancer. This thesis attempts to view the various systems that contribute to cancer's reprogramming of energy metabolism in a broader context, to shed light on the mechanisms that underlie cellular function in health and disease.

Regulation is traditionally viewed as a "governing mechanism" in cellular function, as the system that decides cell's responses to its environment as well as its eventual fate. MicroRNAs have relatively recently secured their position in our concept of the regulatory landscape, as well as in the study of cancer etiology. MiR-21, as one of the archetypical oncomiRs, is quite famous in the latter context, and its involvement in various cancer hallmarks (including cancer metabolism) has been demonstrated in the past. In breast cancer, a cancer type in which miR-21 is known to be strongly overexpressed, we discovered that an enzymatic degradation mechanism specifically operates on certain 3' isoforms of miR-21. In this mechanism, which was also found in a variety of other cell types and species, miR-21+C is first adenylated by the tumor suppressor PAPD5, which renders the microRNA susceptible to a subsequent degradation step by an exoribonuclease, probably PARN (Chapter 2).

While the elucidation of the contribution of individual microRNAs to the cancer phenotype is a budding field of research, "regular" signaling enzymes have had their role in cancer appreciated for quite some time, and in the past,

the identification of the roles of individual proteins within signaling pathways and the roles of these pathways in cancer has proven fruitful on many occasions. We studied the downstream network of the chemokine receptors CXCR4 and CXCR7, which are both activated by the chemokine CXCL12, using a time course of reverse-phase protein array (RPPA) assays. The involvement of chemokine receptors in cancer is well known, but the precise extent of this involvement is unclear, especially in the case of CXCR7. As RPPA is an antibody-based technology, it can distinguish between phosphorylated and non-phosphorylated isoforms of the same signaling enzyme, and their relative levels can be tracked over a time course of CXCL12 stimulation after inhibition of either CXCR4 or CXCR7, or of both. Using RPPA data, we identified p42/44 MAPK phosphorylation as an event downstream of CXCR7 activation, and we then confirmed this in separate Western blotting experiments.

In contrast to the signaling machinery, metabolic enzymes primarily act on small molecules rather than on other enzymes. Therefore, the tools used to study metabolism on a genome-wide scale are vastly different from those used to study signaling, and even from those traditionally used to study metabolism on a smaller scale (namely, kinetic models).

Genome-scale metabolic models (GSMMs), although a coarse-grained approach that purposely ignores various regulatory and kinetic aspects, can shed light on the properties of a large metabolic system based on its structure and on the specifications of its environment. However, their size and format made working with them an exercise in software installation and troubleshooting in addition to the biological challenge at hand. To help overcome this impediment for both researchers (including yours truly) and educators, we developed FAME, an install-free graphical online tool for working with GSMMs, before doing our modeling work.

A subsequent secondary challenge was the interpretation of the vast amounts of results that GSMM analyses produce. Without custom-made computational support, finding the biological phenomenon of interest if one does not know where to look beforehand is an almost Herculean task. Thus, to make the analysis process more human-friendly, we developed hand-drawn maps of metabolism that can be read by computers as well as intuitively interpreted by researchers. By repurposing existing technology to make the map adapt to the model under study as well as integrate with FAME, the results interpretation step of GSMM research should be greatly facilitated.

We then proceeded to study the properties of human metabolism in cancer cells. A very comprehensive reconstruction of human metabolism ("Recon2") was available for just this purpose, but its biomass equation being derived from a combination of non-human species, we first experimentally determined the first human biomass equation for use in genome-scale models. After including the biomass composition of HL-60 human leukemia cells as an objective function in Recon2, we determined the requirements for growth that this biomass equation places on the medium. We discovered that several fatty acids are essential for HL-60 growth, and since the defined part of RPMI1640 medium does not contain them, we separately confirmed their presence in serum-containing medium.